BME SENIOR CAPSTONE PROJECT

PROJECT TITLE: Characterization of Brain Metabolic State under Injury using Two-Photon Microscopy

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ABSTRACT: Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. However, TBI remain difficult to identify and treat in the clinic due to a lack of known biomarkers that can be used as diagnostic and therapeutic targets. To this end, 3D engineered brain tissues seeded with human induced neuronal stem cells (hINSCs) are assessed using multimodal label-free two-photon excited fluorescence (TPEF). TPEF generates endogenous fluorescence from several metabolic co-enzymes and stress-associated cellular products, which are measured by spectral intensity and fluorescence lifetime imaging. We aim to correlate optical measurements with biochemical and metabolomic assays in the context of two major aspects of TBI, glutamate excitotoxicity and oxidative stress. This work will ultimately be used to develop a metabolic model that will use optical measurements to identify biomarkers that are implicated in TBI-associated pathways.

KEYWORDS: Traumatic brain injury, two-photon imaging, metabolic pathways

ELEMENTS OF ENGINEERING DESIGN:

The *design of this project* is the characterization of optical readouts by mapping them to specific metabolic pathways that are affected by injury. This can be achieved with a 3-compartments system: cell culture, imaging , and computational model. The *objectives are set* based on each compartment as follows. First, mono- and co-cultures of brain cells will be performed on a 2D platform. Injury will be induced in cell cultures and its impact will be examined via microscopy, metabolic assays, and mass spectrometry. Finally, computational models will be created to identify affected metabolic pathways from biochemical data. These *objectives can be tested and evaluated* as follows. Once a comprehensive cell culture protocol has been developed; we will only consider our cultures viable if they remain stable at passage 3 and are healthy as observed in baseline imaging readouts, which will be compared to imaging readouts from validated cultures in the lab. Mass spectrometry will follow the validated protocols from the Lee lab and therefore do not require evaluation. Similarly, the acquisition of optical images will be adapted from the imaging protocols of the Georgakoudi lab. We will evaluate successful induction of our injury conditions by asserting that trends of optical readouts and mass spectral data converge, indicating that the experimental treatment successfully induced a consistent metabolic shift. As an additional safety net, results from metabolic assays should agree with the data from mass spectrometry given that both methods measure metabolomics concentrations.

Multiple *engineering principles* are applied in this project with the most prominent one being two-photon microscopy (TPEF) - an imaging modality for injury assessment. Compared to standard fluorescence microscopy, TPEF utilizes a pulsed, non-linear excitation process where 2 photons are used to excite the fluorophore. By lowering the amount of energy needed per photon, TPEF uses a longer wavelength, which generates less tissue damage and penetrates deeper. Notably, sufficient laser intensity for this excitation is only achievable in the focal plane. This restricts the volume of the signal generation as out-of-focus signals from the planes above and below the focal plane of the sample are removed. These characteristics make TPEF depth-resolved, facilitating the imaging of thick and highly scattering specimens like engineered brain tissue (EBT) without the need of slicing or biopsy (non-invasive). For this project, endogenous fluorophores such as FAD and NADH will be used so that the imaging process is label-free where samples can be dynamically examined over time (live imaging).

There are 2 major *realistic constraints*: ethical concerns and translatability of the computational model. Specifically, there are ethical concerns of incurring TBI to human brains or using postmortem samples. As a *solution*, we will use 3D-engineered brain tissues which show pathophysiology observed in an *in-vivo* model [15]. While there are ethical concerns due to the use of human neuronal stem cells, this is necessary to accurately determine if our results are clinically translatable. Additionally, we plan to use a model of brain metabolism at baseline derived from literature because there is no existing TBI metabolic model to our knowledge. Metabolic model source code is difficult to obtain, and models may be designed based on assumptions and conditions specific to the institution which published that model. That is, it may be a non-trivial task to adapt existing models in a way that is relevant for our data. A solution to this problem would be to write our own model based on the key differential equations governing the metabolic processes of interest to us (central metabolism, glutamate-glutamine conversion, and oxidative stress). This would be outside the scope of our capstone, but would be doable by future students.
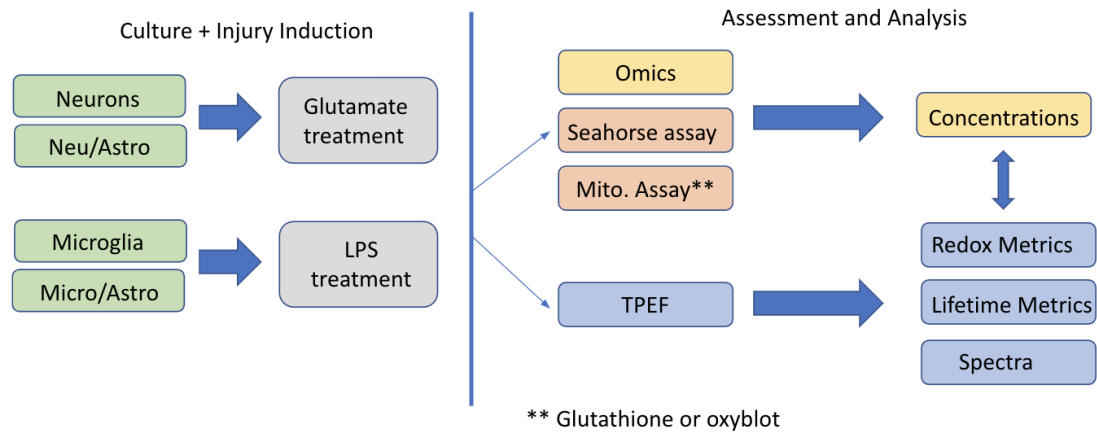
DESIGN FLOWCHART

Figure 1. Schematic overview of experimental plan

INTRODUCTION AND BACKGROUND

Traumatic brain injury is the leading cause of death among individuals under the age of 45 in the US, with an incidence of 1.5 million each year. Beyond fatality, TBI results in severe long-term disabilities, both mentally and physically. [3] Pathophysiologically, Traumatic brain injury can be divided into 2 phases: a primary mechanical impact to the brain followed by secondary biochemical and inflammatory cascades of different types of brain cells. The two major biochemical cascades that we plan to characterize are oxidative stress and glutamate excitotoxicity [9]. Following the injury, an influx of excess calcium ions into the mitochondria triggers the production of reactive oxygen species (ROS) and free radicals. These molecules depolarize the mitochondrial inner membrane, disrupting the electron transport chain and inhibiting the oxidative phosphorylation process. This deprives the nerve cells from ATP and facilitates apoptosis. In junction with oxidative stress, glutamate and aspartate neurotransmitters accumulate at the synapses as the impaired glutamate transporters fail to recycle excess glutamate from injured neurons. These molecules bind to NMDA and AMPA receptors that promote calcium, potassium, and sodium uptake. Cell depolarization triggers downstream cascades that prolong the effect of oxidative stress. From these observations, it can be said that the biochemical pathways involved in secondary injury are highly complex and intertwined. Despite ongoing research, the understanding of its mechanisms and consequences remains incomplete. Notably, secondary injury can develop over a long period of time, increasing the severity of the original injury. In other words, patients with mild TBI can suffer neurological problems and long-term disability months after the injury. For this reason, patients with mild TBI have no initial symptom and, therefore, are often undiagnosed, preventing early treatment. Consequently, the *long-term objective* is to develop a technique that can diagnose TBI on a molecular level, which is the biochemical cascade of secondary injury.

To examine the long-term cellular effects of mild TBI, the 3D engineered brain tissues (EBT) of neurons and glial cells (astrocytes and microglia) are injured using the controlled cortical impactor (CCI), mimicking a mild blast TBI. The EBT model, while a simplified human brain, still undergoes most of the complex secondary response following the impact and, thus, can be used to develop diagnostic and treatment frameworks for TBI. However, with CCI, it is

challenging to completely characterize this model due to the evolving complex cellular environment and unpredictable changes arising from the interactions between multiple cell types. Thus, we propose to examine TBI via its constituents by introducing a specific secondary injury to 2D brain cell cultures and study the cellular metabolic interactions and environments in a controlled manner.

Two-photon excited fluorescence (TPEF) can then be used to assess the functional and morphological changes of the injured brain cells by obtaining the metrics of cellular metabolic function. TPEF detects autofluorescent signals from several key biomolecules, namely FAD, NADH, LipDH, and lipofuscin. The former three are metabolic co-enzymes which are implicated in most metabolic perturbations, and lipofuscin is a complex of fluorescent proteins and lipids that accumulates under cellular stress conditions. These endogenous fluorophores can be analyzed using computational techniques that reveal underlying concentration-based and metabolic shifts in the samples: redox ratio, mitochondrial clustering, phasor analysis, and spectral deconvolution.

Redox ratio is the relative ratio of glycolytic to oxidative metabolism. In brief, it is computed by obtaining a "NADH image" (755ex/460em) and a "FAD image" (860ex/525em) and dividing them according to the formula (NADH/(NADH+FAD)). *Mitochondrial clustering* is the extent of mitochondrial fractionation, which occurs in response to ROS accumulation. It is computed by segmenting and cloning mitochondrial regions in an image. Then, the power spectral density of the cloned image is computed, which determines the image frequency. Highly fractionated mitochondria will have a high frequency, and vice versa. *Phasor analysis* is a technique to obtain fit-free visualizations of FLIM images with overlapping concentrations of lifetimes over different pixels. In brief, time-series fluorescence lifetime data is sine and cosine transformed, giving two coordinates g and s that correspond to the lifetime, *tau*, of the fluorescent decay. Any one *tau* localizes on a circular plot (see Fig. 2). The localization of the (g, s) coordinate pair for any given pixel is determined by the linear combination of different *tau* values constituent in the pixel. A fluorophore's binding environment affects its lifetime, but its concentration does not (i.e. higher concentrations of a single fluorophore simply cause a shift in the phasor distribution towards that fluorophore's lifetime). As such, by assessing the overall phasor distribution, conditions such as shifts in relative concentrations of fluorophores and shifts in fluorophore binding configuration can be observed. *Spectral constituents* are obtained from the overall spectral intensity curve via non-negative matrix factorization. In this method of spectral deconvolution, the user specifies the number of total constituents and the model computes optimal concentrations of non-negative vectors and weights that minimize the error (residual) from the overall spectrum. In this way, concentrations and emission spectra of constituent fluorophores are determined.
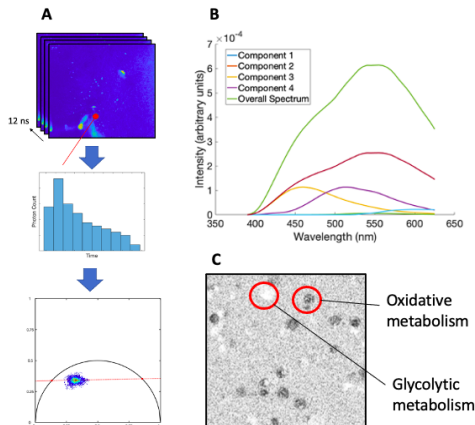

Figure 2. Description of lifetime phasor analysis (a), redox ratio (b), and spectral deconvolution (c).

Compared to state-of-the-art diagnosis procedures like MRI, TPEF is more sensitive to cellular-level metabolic shifts. However, TPEF fails to detect non-fluorescence metabolites such as lactate. Additionally, while it is known that an increase in redox ratio correlates to an increase in glycolytic metabolism and vice versa, conducting redox ratio studies in conjunction with exact biochemical measurements will allow us to quantify how shifts in oxidative and glycolytic metabolism affect our optical readouts. This lack of specificity is a *critical roadblock* for using TPEF to study injured brain metabolism. Consequently, the *specific goal* is to characterize and map optical metrics to specific altered metabolic pathways predicted by a metabolic computational model. The *central hypothesis* for identifying the pathways is to input relevant biochemical metrics from assays and mass spectrometry to the computational model.

Two-photon imaging is a commonly used neuroimaging technique due to its high depth penetration and potential for metabolic sensitivity, so many groups researching the impact of TBI or other neurodegenerative diseases choose to use two-photon imaging. Additionally, biological assay and mass spectrometric methods are well-investigated in the context of TBI [1-4]. Therefore, **the novelty of this project lies in correlating a non-invasive, label-free method (TPEF) with these invasive methods for the eventual use of optical methods alone for a diagnostic TBI model**. A non-invasive, label-free platform for the assessment of TBI does not exist to our knowledge. As such, the novelty of this study depends on the identification of TBI biomarkers, not just the development of a two-photon platform to study brain injury.

In general, we propose to formulate a relationship between output molecular concentrations from assays and optical readouts via a computational model. We *hypothesize* that, under different perturbed or injured conditions, the trend in optical readouts and molecular concentration will be different since different metabolic mechanisms are involved. Therefore, we can say that a specific trend in optical readouts will be characteristic of a set of output concentrations and from the computational mode, specific altered metabolic pathways. This will allow us to characterize the optical readouts for a specific injury condition.

This work will fit within the research being performed by Ph.D. candidate Yang Zhang and postdoctoral scholar Maria Savvidou in the ODDET Lab. So far, they have acquired multimodal (spectral, fluorescence lifetime, and intensity) two-photon images of the EBT model under injury and control conditions. *Our work will focus on the specific characterization of aspects of TBI in simpler, controlled culture and injury settings in order to better understand the correlation between cellular shifts and optical readouts in the EBT data.*

SPECIFIC AIMS, METHODS, AND RESULTS

Specific Aim 1 (SA1): *Assessment of controlled secondary injury in monoculture*
Thus far, injury to the 3D engineering brain tissue is induced in a non-specific manner via a stereotaxic impactor. In order to study a targeted metabolic cascade (secondary injury) of TBI such as glutamate excitotoxicity, specific metabolic perturbations must be introduced and changes to relevant metabolic pathways can be detected. However, since this study has not been done previously, it's important that we perform a preliminary study on the monoculture to optimize treatment concentrations and refine detection methods.
Study 1: Induce secondary injuries to monoculture of microglia
Excess lipopolysaccharide (LPS) can be added to the microglia monoculture to trigger oxidative stress. The rationale for using microglia as a primary cell line is because LPS interacts with transmembrane signaling receptor toll-like receptor 4 which is expressed primarily on

microglia. The range of LPS concentration that will be tested is 4-12 μg/mL, with an increment of 2 μg/mL, because previous research has shown that maintaining this range is sufficient to induce oxidative stress and maintain cell survival rate between 70% to 80% [10]. Previous work has been done by members of our lab to verify that our protocol successfully induces LPS uptake in microglia. We will use 3 well replicates for each concentration at each timepoint, and one control set. To assess the cultures, we will use multimodal TPEF (see Data Acquisition) at 12 and 24 hours. At the last time point, mass spectrometry will be performed on the injured condition and the control condition.

There are 2 *success measures* to indicate that the treatment is effective. First, we will compare mass spectra from the injured and baseline conditions to assess that there is a significant difference between the conditions. This can be done using a linear discriminant analysis that should have a 90% classification rate between the spectra. Second, we will validate that the optical readouts trend consistently towards glycolysis, as it is well-known that oxidative stress causes a shift to glycolytic metabolism. The phasor distribution should move to the bottom right to indicate increased free NADH, redox ratio should increase, and spectral constituents should have an increased NADH concentration; all of these validations are based on previous work on oxidative stress in the ODDET lab.

Study 2: Induce secondary injuries to monoculture of neurons

Glutamate excitotoxicity can be introduced by adding excess glutamate in a HEPES-buffered salt solution (containing 120mm NaCl, 5.4mm KCl, 0.8mm MgCl2, 1.8mm CaCl2, 15mm glucose, and 20 mm HEPES at pH 7.4) to the neuronal monoculture. Based on previous study of neuronal excitotoxic injury, the range of glutamate concentration that will be tested is 2-5 μmol/L, with an increment of 0.5 μmol/L [11]. We will expose the monocultures to the glutamate solution for ten minutes and then aspirate the glutamate solution, as performed in previous studies [12]. We will assess the cultures using multimodal TPEF (see Data Acquisition) at 6, 12 and 24 hours. Well replicates and mass spectrometry are as in Study 1.

The *success measures* are the same as in Study 1. Additionally, the mass spectra at the end time point should have a glutamine peak significantly greater than the baseline (assessed with Student's t-test of normalized peak height). This is because extracellular glutamate is converted to glutamine, which can diffuse back to the postsynaptic neuron for recycling.

Data acquisition The effect of the induced perturbations will be examined via mass spectrometry and two-photon excitation microscopy. It is critical that both acquisitions occur at the same time point for each perturbation to ensure the readouts are correlated.

Mass Spectrometry: Relevant metabolites (based on previous research) – glutamate, glutamine, creatinine, and decanoic acid – will be tagged so that their concentration changes can be detected with mass spectrometry [13]. The *goal* of using mass spectrometry in both studies is to identify metabolites that are heavily upregulated or downregulated after injury induction and verify that the detection method is suitable. To achieve this, we will conduct mass spectrometry at the start and end time point.

Imaging: We will perform multimodal (spectral intensity and fluorescence lifetime) TPEF acquisition according to standarding imaging protocols in the Georgakoudi lab. We will measure spectral intensity at 755nm, 860 nm and 910nm excitation, detecting on a multi-wavelength PMT every 10 nm from 490 to 630 nm. We will measure fluorescence lifetime at the same excitations but detect only at 460 and 525 nm using a hybrid detector and a PicoQuant TCPSC module which allows for the high temporal resolution needed for fluorescence lifetime imaging.

Data Analysis

Imaging: Redox ratio, mitochondrial clustering, lifetime phasor distributions, and spectral constituents are obtained by custom-written MATLAB code.

Mass Spectrometry:

Clustering analysis using Pearson correlation will identify tagged metabolites that are not heavily upregulated or downregulated and thus, can be removed in future studies. Then, ANOVA with a post-hoc Student's t-test will be used to determine treatment concentration, for each study, that results in significant concentration change of desired metabolites.

Potential Pitfalls and Alternatives:

Glutamate excitotoxicity is a fast-acting and sensitive process; also, unlike LPS induction, we have not previously performed this protocol in the lab. Therefore, it is possible that we will encounter difficulties inducing sufficient glutamate uptake or dealing with unprecedented reactions to the addition and removal of exogenous glutamate. Alternatively, a previous study has shown that 200 μM of DL-TBOA is sufficient to inhibit the NMDA glutamate receptor and, thus, induce glutamate excitotoxicity in human neurons derived from embryonic cells without extraneous glutamate [14].

Specific Aim 2 (SA2): *Assessment of controlled secondary injury in neuron-microglia and neuron-astrocyte co-cultures*

Study 1: Induce secondary injuries to co-culture of neuron-microglia

Oxidative stress can be induced by adding optimal concentration of LPS that has been determined in SA1 study 1. LPS concentrations must maintain cell survival rate above 80% at the last time point of data acquisition. All experimental design aspects remain the same as SA 1, but we will use 5 well replicates at only one concentration. The *success measures* are the same as those for SA1 study 1.

Study 2: Induce secondary injuries to co-culture of neuron-astrocyte

Glutamate excitotoxicity can be induced by adding optimal concentration of glutamate or DL-TBOA that has been determined in study 2 of SA1. All experimental design aspects remain the same as SA 1, but we will use 5 well replicates at only one concentration. The *success measures* are the same as those for SA1 study 2.

Data acquisition

Mass spectrometry: Tagged mass spectrometry at the start and end time point will be used to detect changes in metabolite concentrations as described in SA1; inclusion or removal of certain metabolites may be performed based on findings from SA1 that certain metabolites are redundant in terms of injury response or that certain key aspects of the injury response are not captured by the current metabolites.

Metabolic Assays: Metabolic assays and imaging acquisition will be done in parallel to ensure readouts are tightly coupled. The Seahorse assay (Agilent Technologies) is a standard ATP production assay that measures relative levels of oxidative and glycolytic metabolism. Additionally, a glutathione/GSH assay (Sigma Aldrich) will be used to assess oxidative stress and glutamate-associated metabolic effects. Glutathione is upregulated under oxidative stress and also contributes to glutamate uptake and metabolism in TBI, so this critical effector in the glutamate injury pathway will be monitored at multiple timepoints simultaneously with imaging.

Overall, the use of metabolic assays in combination with mass spectrometry (a) strengthens the most critical findings related to metabolic stress by providing an additional data verification (b) allows for a multi-timepoint biochemical assessment that can be parallelized with the imaging sessions, as opposed to mass spectrometry which can only be performed at one timepoint due to experimental burden.

Optical imaging: As in SA1, TPEF detects signals from FAD, NADH, LipDH, and lipofuscin, which can be analyzed to obtain redox ratio, phasor plots, and spectral deconvolution.

Data Analysis: Data analysis is as in SA1.

Specific Aim 3: *Develop a computational metabolic model that predicts injury pathway activation based on biochemical readouts.*

Study 1: Develop a basic computational metabolic model for TBI cultures

We will obtain relevant brain metabolism computational models from the literature (neuron/astrocyte/microglia metabolism, oxidative stress models, and injury models) and modify them for the purposes of our study by adjusting concentration conditions based on our mass spectrometry results from SA 1 and SA 2. Molecules involved in the model that are not present in our spectrometric results will be treated as assumed constants based on literature values. The completed model will include central metabolism, detoxification of reactive oxygen species, and the glutamate-glutamine cycle. It will be able to predict the relative level of pathway activation (ex. glycolytic vs. oxidative metabolism) based on the input concentrations of downstream metabolites, as obtained from mass spectrometry. We will validate the accuracy of our metabolic model by performing mass spectrometry on baseline cultures and assessing the similarity of our predicted pathway results with results obtained from the metabolic models from which our model was derived. This is to ensure that in integrating multiple models, we preserved the integrity of each individual model.

Study 2: Use the metabolic model to predict injury pathway activation

From SA 1 and SA 2, we will have imaging data from secondary injury at multiple timepoints and corresponding mass spectrometry data from the final timepoint. We can use the metabolite concentrations and the metabolic model to predict levels of pathway activation under injury conditions, and then correlate those pathway activations with the optical readouts from those same conditions. While the metabolic model does not output pathway activations, it predicts concentrations of upstream effectors that would cause observed downstream metabolite concentrations. By associating upstream effectors to particular injury pathways, we can estimate the pathway activations under different injury conditions.

Data Acquisition: This SA uses data obtained in SA 2.

Data Analysis: We will correlate optical readouts with pathway activations in LPS and glutamate conditions using a linear regression across timepoints. We will train on 4 out of the 5 replicates from SA 1 and test on the other using a k-fold cross-validation. The regression will be validated based on a 0.85 Pearson correlation, and a statistically significant improvement for testing data from the wrong pathway to the correct pathway (i.e. glutamate optical readouts correlated against LPS concentrations should correlate significantly worse than glutamate readout correlated against glutamate concentrations).

Potential Pitfalls and Alternatives: If the linear regression cannot be used to separate the injury pathways, we will consider machine learning approaches. This will require much larger-scale data acquisition and would likely go beyond the scope of this project. However, future students could improve replicates of this data across more concentrations. Then, they could use a logistic regression based on imaging and mass spectrometry raw data to directly classify imaging data as either glutamate-resultant or LPS-resultant.
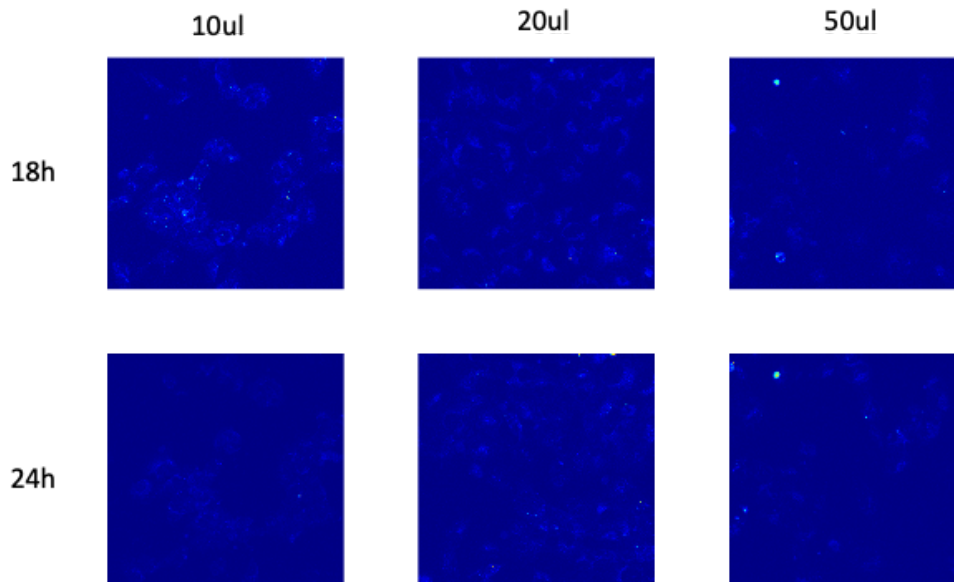
PRELIMINARY RESULTS

Figure 3. Optimization parameters for LPS induction in microglia.

Images of increasing concentrations of LPS in microglia at 755 nm excitation and summed spectral emission (490-630 nm) are shown in Figure 3. The same regions were imaged at 18 hours and 24 hours post-induction. Images are normalized to maximum intensity. LPS localization, indicated by the saturated bright spots, is present at all concentrations and time points but is most noticeable at 50 ul at 18h, and at 20 and 50 ul at 24h. The same regions, particularly at 50ul, accumulate more LPS as a function of time. Additionally, at high concentrations, particular regions seem to preferentially uptake LPS, which is undesirable for studies of multiple cell regions. However, due to a lack of replicates – this pilot was done at 6 concentrations (3 concentrations are not shown) for 2 timepoints with only 1 sample per condition – no conclusions can be made about optimal concentrations, and optical readouts cannot be analyzed with statistical significance.

DISCUSSION AND FUTURE WORK

In the first semester of the project, we hope to successfully develop monocultures for microglia, then neurons. We may run into issues with the protocol, which would push back the

computational part of the project. In order to account for that, we will start working on an outline for the computational model ahead of time using external resources.

In the second semester of the project, we aim to develop co-cultures for further imaging and metabolic analysis. With monoculture data from the first semester, we will be able to link the optical readouts with the metabolic data using statistical tools on MATLAB. We can compare this correlation in data to our computational model framework and adjust it as needed. Ideally, the researched functions we collected will accurately reflect our results.

The goal of our project is to correlate optical measurements with biochemical and metabolomic assays. Understanding this correlation will allow us to develop algorithms to predict biochemical and metabolomic data purely from optical readouts. This is valuable because it reduces the time and cost required to see the metabolic effects of TBI on brain cells. Culturing cells and completing assays can cost thousands of dollars over the span of many months. The development of this algorithm will make it possible to do TBI research in a shorter timespan and a smaller budget. This broadens the availability of TBI research to labs which do not have the resources to do cell culturing long-term. This also helps address the morality of using engineered brain tissue because it reduces the need for handling it.

UNIFYING FIGURE

BME7: Characterization of Brain Metabolic State under Injury using Two-Photon Microscopy (F'22)
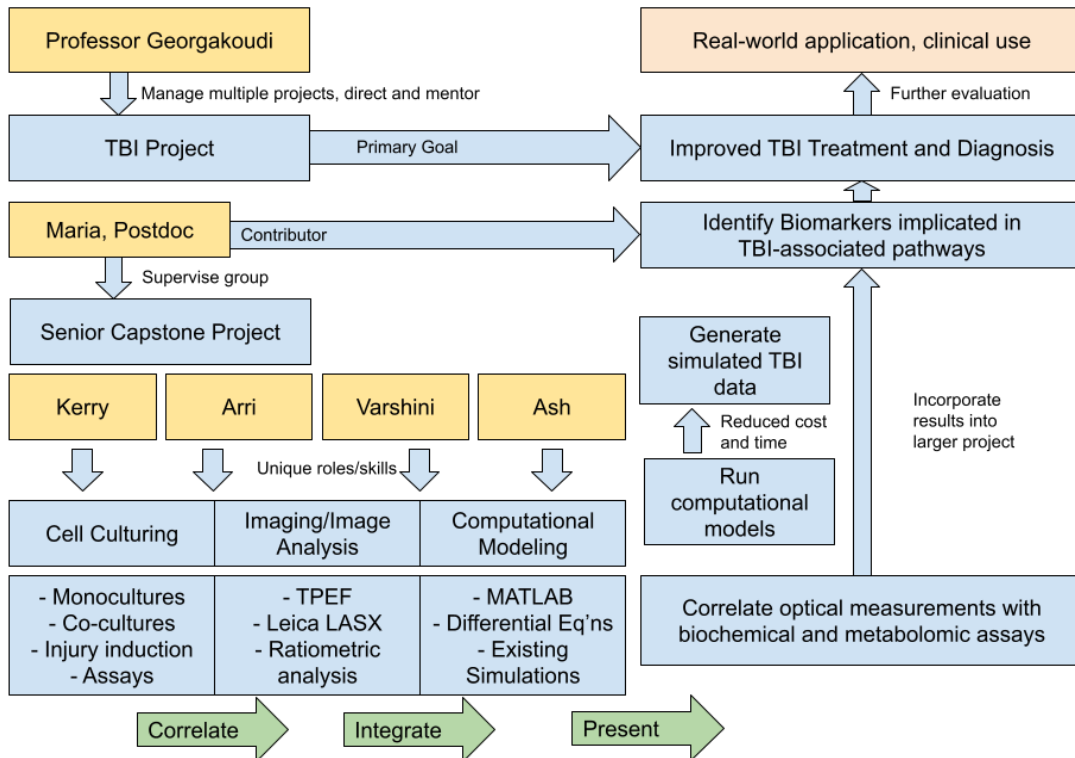
Supp. Figure 1. The unifying figure describes a simplified hierarchy of the Georgakoudi lab, the roles and objectives of our project, the contribution of our project to the main project objective, and real world implications.

INDIVIDUAL CONTRIBUTIONS
The individual contribution of each group member to the project will be as follows:

1. Computational modeling includes compiling existing code about brain metabolic pathways affected by TBI, extracting valuable functions from external sources and writing code in MATLAB to analyze patterns in data found through co-cultures and imaging. Examples of results are an algorithm that simulates neuron-astrocyte interaction and graphs the simulated data.
2. Imaging includes using multi-modal two-photon microscopy to observe engineered brain cell activity after simulated concussions. Imaging will be done on monocultures first(microglia, neurons, astrocytes), then co-cultures in the second semester if there is enough time.
3. Image analysis includes ratiometric analysis of different detector channels, phasor analysis, data processing, cellular segmentation and automatic annotation. This data will be correlated with cell culturing data.

4. Cell culturing includes developing co-cultures and writing the secondary injury protocol. We are starting by culturing microglia because there exists an established protocol. The goal is to culture neurons and co-cultures as well and collect metabolic data for correlation to image analysis results.

Ash will be responsible for computational modeling and assisting with image analysis under guidance from Varshini.

The first task in computational modeling is to compile existing code that models glutamate excitotoxicity, lipofuscin, general brain metabolics and any other pathways relevant post TBI. These models will likely be used on MATLAB in the form of differential equations. Ideally, the models can successfully model chemical outputs given optical readouts.

The previous senior capstone project from the Georgakoudi lab tried to tackle the creation of a machine learning algorithm. Therefore, it would be valuable to communicate with Aonkon Dey, the student in charge coding the algorithm, what limitations and challenges they have encountered during their project. This means compiling more code and using limitations to help decide what assays are appropriate.

Varshini will be responsible for guiding team members through higher-level computational work and imaging/image analysis. She will be working primarily on imaging/image analysis as well as coding for the computational model.

As the one most experienced with the Georgakoudi lab, Varshini will be the main point of contact with the post-doc, Maria, who will further instruct Kerry and Arri in how to culture engineered brain tissue cells. Additionally, she will be able to connect relevant resources from the Georgakoudi lab to our project such as existing protocols and models.

After meeting with our post-doc this week, Varshini has been doing research on alternate assay techniques for measuring glycolysis in neurons because the seahorse assay is located in Grafton. There may be additional issues with using assays and cell culturing because the timespan and cost extends beyond both BME7 and 8. We need to talk to Maria and Professor Georgakoudi about more alternative paths and how we can have more guidance rather than starting the experiment from scratch.

Kerry will be responsible for imaging and cell culturing. She will be familiarizing herself with two-photon microscopy and using her experience in cell culturing to guide Arriety.

A part of cell culturing is developing the protocol. Kerry will be receiving Microglia cell culturing instruction alongside Arri by post-doc Maria from the Georgakoudi lab. In the meantime, she used available protocols and online papers to include well numbers, replicates, time points, duration of chemical exposure, correlating measurements and number of trials into the draft protocol.

Arrietty will be responsible for developing co-cultures and writing the secondary injury protocol. She will also be using her experience in the lab to help with image analysis.

Arri has been using her existing knowledge of computational modeling to find models for TBI chemical pathways. This includes compiling external resources, using her summer research and reading her BME6 paper.

Working closely with Varshini, Arri is also communicating with Maria and the professor about possible alternatives for our project and training for the use of new assays. She has also been further assisting with finding computational models that are relevant to the pathways we are studying.

All members of the project are completing the training required to work in the lab. This includes BSL2, baseline eye exam, laser training, microscopy training, etc. Overall, we are slightly off-track because the computational models are harder to implement than we anticipated. Additionally, TBI cell cultures require additional training from the post-doc who has a busy schedule. After the training, we will be able to better define challenges, alternatives and specifics (timestamps, replicates, etc.). We need to consider an alternative for the seahorse assay because the location for the assay is in Grafton. We need to optimize the concentrations before culturing which will take time and push back the project. Due to the scale of our experiments and the inexperience of our lab, we will be consulting our supervisor, professor Georgakoudi and the professors for BME7 for advice on getting results in a timely manner.

REFERENCES

1. Haenseler, W. *et al.* A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response. *Stem Cell Reports* **8**, 1727–1742 (2017).
2. Chapman, G. A. *et al.* Fractalkine Cleavage from Neuronal Membranes Represents an Acute Event in the Inflammatory Response to Excitotoxic Brain Damage. *J. Neurosci.* **20**, RC87–RC87 (2000).
3. Dorsett, C. R. *et al.* Glutamate Neurotransmission in Rodent Models of Traumatic Brain Injury. *J Neurotrauma* **34**, 263–272 (2017).
4. Wu, Y.-H. *et al.* In Vitro Models of Traumatic Brain Injury: A Systematic Review. *Journal of Neurotrauma* **38**, 2336–2372 (2021).
5. Robertson, J. M. Astrocyte domains and the three-dimensional and seamless expression of consciousness and explicit memories. *Medical Hypotheses* **81**, 1017–1024 (2013).
6. Bylicky, M. A., Mueller, G. P. & Day, R. M. Mechanisms of Endogenous Neuroprotective Effects of Astrocytes in Brain Injury. *Oxidative Medicine and Cellular Longevity* **2018**, 6501031 (2018).
7. Kim, O. D., Rocha, M. & Maia, P. A Review of Dynamic Modeling Approaches and Their Application in Computational Strain Optimization for Metabolic Engineering. *Frontiers in Microbiology* **9**, (2018).
8. Oschmann, F., Berry, H., Obermayer, K. & Lenk, K. From in silico astrocyte cell models to neuron-astrocyte network models: A review. *Brain Research Bulletin* **136**, 76–84 (2018).
9. Ng, S. Y. and A. Y. W. Lee. "Traumatic Brain Injuries: Pathophysiology and Potential Therapeutic Targets." Front Cell Neurosci **13**: 528, (2019).
10. Shi, H., et al. "The in vitro effect of lipopolysaccharide on proliferation, inflammatory factors and antioxidant enzyme activity in bovine mammary epithelial cells." Anim Nutr **2**(2): 99-104, (2016).
11. Mark, L. P., et al. "Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging." AJNR Am J Neuroradiol **22**(10): 1813-1824, (2001).
12. Chapman, G. A., et al. "Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage." J Neurosci **20**(15): RC87, (2000).
13. Posti JP, Dickens AM, Orešič M, Hyötyläinen T, Tenovuo O. Metabolomics Profiling As a Diagnostic Tool in Severe Traumatic Brain Injury. Front Neurol. doi: 10.3389/fneur.2017.00398. (2017)
14. Gupta, K., et al. "NMDA receptor-dependent glutamate excitotoxicity in human embryonic stem cell-derived neurons." Neurosci Lett 543: 95-100. (2013).
15. Liaudanskaya, Volha et al. "Modeling Controlled Cortical Impact Injury in 3D Brain-Like Tissue Cultures." Advanced healthcare materials vol. 9,12 (2020).